Docket No.: 067234-0056 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK EXAMINER BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of CHEE, Mark S., et al.	Customer Number: 41552
Application No.: 09/513,362	Confirmation Number: 7034
Filed: February 25, 2000	Tech Center Art Unit: 1637
Examiner: STRZELECKA, T.	

For: NUCLEIC ACID SEQUENCING USING MICROSPHERE ARRAYS

APPEAL BRIEF TRANSMITTAL

CERTIFICATE OF ELECTRONIC TRANSMISSION

I hereby certify that this correspondence is being electronically-transmitted to the United States Patent and Trademark Office on **December 14, 2007.**

/Carrie Hines/

Carrie Hines

Mail Stop Appeal Brief - Patents
Commissioner for Patents
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Sir:

Submitted herewith is Appellant's Appeal Brief in support of the Notice of Appeal filed on November 30, 2007. To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due under 37 C.F.R. §§ 1.17 and 41.20, and in connection with the filing of this paper, including extension of time fees, to Deposit Account 502624 and please credit any excess fees to such deposit account.

Respectfully submitted,

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I. REAL PARTY IN INTEREST

The real party in interest is ILLUMINA, INC.

II. RELATED APPEALS AND INTERFERENCES

Appellants are unaware of any related Appeal or Interference. Pending application United States Serial No. 10/264,574, filed October 4, 2002, which is a continuation-in-part application of the above-captioned application, is presently involved in an interference with U.S. Patent No. 6,858,412. The counts of the Interference are unrelated to the subject matter claimed in the above-captioned application now being appealed.

STATUS OF CLAIMS

Claims 1-21 were initially presented in this application. Claims 22-50 were added during prosecution. Claim 39 has been previously cancelled. Claims 1-38 and 40-50 presently stand finally rejected in this application. It is from the final rejection of claims 1-38 and 40-50 that this appeal is taken.

III. STATUS OF AMENDMENTS

No amendment has been filed subsequent to the Final Office Action mailed on May 30, 2007.

IV. SUMMARY OF CLAIMED SUBJECT MATTER

The following support for all claims is cited to the page:lines of the specification filed on February 25, 2000.

Independent claim 1 is directed to a method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, the second domain comprising a plurality of target positions. The method of claim 1 comprises providing an array comprising a substrate with a surface comprising discrete sites and a population of microspheres comprising at least first and second subpopulations, distributed at discrete sites on the surface of a substrate; and an enzyme attached at the discrete sites, wherein the enzyme is used to generate a signal from pyrophosphate; providing a first hybridization complex comprising the first domain of a first target sequence and a first sequence primer, wherein the first hybridization complex is attached to the first subpopulation; providing a second hybridization complex comprising the second domain of a second target sequence and a second sequence primer, wherein the second hybridization complex is attached to the second subpopulation; simultaneously extending the first and second primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primers, respectively; detecting the release of pyrophosphate (PPi) with the enzyme attached at the discrete site within a common reaction chamber of the simultaneous extensions to determine the type of the first nucleotide added onto the first and second primers, respectively; and determining sequences for the plurality of target nucleic acids. Claim 1 is supported throughout the specification as originally filed, for example, by claim 1, by Figure 1, and at pages 12:12-16, 13:37, 14:34-15:24, 24:34, 26:16-24, 30:16-34, and 31:5-36.

Claim 10 is directed to a method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, the second domain comprising a plurality of target positions. The method of claim 10 comprises providing a first hybridization complex comprising a first target sequence and a first sequencing primer that will hybridize to the first domain of the first target sequence; providing a second hybridization complex comprising a second target sequence and a second sequencing primer that will hybridize to the second domain of the second target sequence, wherein the first and second sequencing primers are covalently attached to microspheres distributed at discrete sites on a surface of a substrate, the discrete sites having an attached enzyme used to generate a signal from pyrophosphate; determining the

identity of a plurality of bases at the target positions, wherein the determining comprises simultaneously extending the first and second sequencing primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primers, respectively; and detecting the release of pyrophosphate (PPi) with the enzyme attached at the discrete sites within a common reaction chamber of the simultaneous extensions to determine the type of the first nucleotide added onto the first and second sequencing primers, respectively. Claim 10 is supported throughout the specification as originally filed, for example, by claim 10, by Figure 1, and at pages 13:37, 24:34, 30:16-34, and 31:5-36.

Claim 18 is directed to a kit for nucleic acid sequencing comprising a composition comprising a substrate with a surface comprising discrete sites; a population of microspheres distributed on the sites; wherein the microspheres comprise different capture probes, wherein the array is configured for simultaneous contact of the different capture probes with a common reaction chamber; and an enzyme attached at the discrete sites, wherein the enzyme is used to generate a signal from pyrophosphate; a first extension enzyme; and dNTPs. Claim 18 is supported throughout the specification as originally filed, for example, by claim 18, and at pages 12:12-16, 13:37, 24:34, 30:16-34, and 31:5-36.

Claim 34 is directed to a method of sequencing a genome comprising amplifying a genome, thereby obtaining a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions. The method of claim 34 encompasses providing an array comprising a substrate with a surface comprising discrete sites; a population of microspheres comprising at least a first and second subpopulation, distributed at said discrete sites; and an enzyme attached at said discrete sites, wherein said enzyme is used to generate a signal from pyrophosphate; hybridizing sequencing primers to said first domains of said target sequences, wherein said hybridization complexes are attached to said microspheres; simultaneously extending said primers by the addition of a first nucleotide to a first detection position using a first enzyme to form an extended primer; and detecting the release of pyrophosphate (PPi) with said enzyme attached at said discrete sites within a common reaction chamber of said simultaneous extensions to determine the type of said first nucleotide added onto said primers; and determining sequences for said genome. from said flow cell following said simultaneously extending said primers. Support for claim 34 can be found at pages 1:23-25, 5:32-36, 6:3-7, 8:7-9, 12:12-16, 14:34-15:24, 49:35-50:1 and claim 1 as filed.

V. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Whether claims 1-16, 22-27, 31-38, 40-42, 44-47, 49 and 50 are unpatentable under 35 U.SC. § 103(a) over Rothberg et al., U.S. Patent No. 6,274,320, and Walt et al., U.S. Patent No. 6,327,410.

2. Whether claim 17 is unpatentable under 35 U.SC. § 103(a) over Rothberg et al., U.S. Patent No. 6,274,320, and Walt et al., U.S. Patent No. 6,327,410, as applied to claim 10 above, and further in view of Nyren et al., WO 98/13523.

3. Whether claims 18, 19, 28-30, 43 and 48 are unpatentable under 35 U. C. 103(a) over Rothberg et al., U.S. Patent No. 6,274,320, Walt et al., U.S. Patent No. 6,327,410, Nyren et al., WO 98/13523, and Stratagene Catalog (1988, at page 39).

3. Whether claims 20 and 21 are unpatentable under 35 U. C. 103(a) over Rothberg et al., U.S. Patent No. 6,274,320, Walt et al., U.S. Patent No. 6,327,410, Nyren et al., WO 98/13523, and Stratagene Catalog (1988, at page 39), and further in view of Ross et al., WO 91/06678.

VI. ARGUMENT

Appellants separately argue the patentability of the following claims as disclosing and claiming separate and distinct embodiments with distinctly different methods and kits: (1) Claims 1-17, 22-27, 31-38, 40-42, 44-47, 49 and 50; and (2) Claims 18-21, 28-30 and 43.

A. The Examiner's Rejections Under 35 U.S.C. § 103(a)

In rejecting claims 1-16, 22-27, 31-38, 40-42, 44-47, 49 and 50 as unpatentable over Rothberg et al., U.S. Patent No. 6,274,320 ("Rothberg et al."), and Walt et al., U.S. Patent No. 6,327,410 ("Walt et al."), the Examiner has made the following assertions on the record, inter alia, in Office Actions mailed 12/12/06 and 5/30/07, to meet the Examiner's burden of establishing a prima facie obviousness rejection:

(1) Regarding claims 1-16, 22-27, 31-38, 40-42, 44-47, 49 and 50, the Examiner has taken the position that Rothberg et al. teach each element of the claimed methods of sequencing a plurality of target nucleic acids *except* the use of microspheres to attach sequencing reactants to the surface of the fiber optic bundle. Office Action mailed 12/12/06: pp. 8-12; Office Action mailed 5/30/07: pp. 5-10.

(2) The Examiner asserts that the alleged deficiency of Rothberg et al. with regard to base claims 1, 10 and 34, is cured by Walt et al., which is alleged to teach microsphere-based analytical chemistry system in which the microspheres are distributed on a fiber optic bundle that comprises discrete sites into which at least two subpopulations of micro spheres are distributed. Office Action mailed 12/12/06: pp. 12-13; Office Action mailed 5/30/07: pp. 10-11. According to the Examiner, each of the microspheres described by Walt et al. comprises a bioactive agent and an optical signature which allows identification of the bioactive agent. The microspheres are allegedly randomly distributed on the array and the bioactive agent that is allegedly attached to the microsphere is a nucleic acid probe. The array is allegedly used for sequencing. The Examiner further alleges that Walt et al. teaches elements of claims 22, 31, 32, 35 and 38 that relate to the mcirospheres and are not taught by Rothberg et al..

(3) The Examiner claims that it would have been prima facie obvious to one skilled in the art to use the microspheres of Walt et al. distributed over the surface of a fiber optic sensor in the sequencing method of Rothberg et al. allegedly because Walt et al. provide a method of generating large fiber optic arrays and because microsphere-based chemistry systems allow synthesis of bioactive agents to be separated from their placement on an array. (e.g., see Office Action mailed Nov. 28, 2005 at pp.10-11, and Advisory Action mailed May 1, 2006 at p.2). The motivation to use microspheres immobilized on a surface of fiber optic bundle rather than nucleic acids or proteins immobilized directly on the surface of the fiber optic bundle allegedly comes from Walt et al. since it allows for fast generation of large fiber optic arrays. Office Action mailed 12/12/06: pp. 11-12; Office Action mailed 5/30/07: pp. 13-14.

In rejecting claim 17 as unpatentable over Rothberg et al., supra, and Walt et al., supra, as applied to claim 10, and further in view of Nyren et al., WO 98/13523 ("Nyren et al."), the Examiner has made the following assertions on the record, inter alia, in Office Actions mailed 12/12/06 and 5/30/07, to meet the Examiner's burden of establishing a prima facie obviousness rejection.

(1) The Examiner alleges that Rothberg et al. teach pyrosequencing using nucleotides, but admits that Rothberg et al. do not teach protected nucleotides. Office Action mailed 12/12/06: p.12; Office Action mailed 5/30/07: p.15.

(2) The Examiner further asserts that Nyren et al. teach pyrosequencing using 3 ' protected nucleotides. Office Action mailed 12/12/06: p.12; Office Action mailed 5/30/07: p.15.

(3) The Examiner concludes that it would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used the 3' protected nucleotides of Nyren et al. in the method of pyrosequencing of Rothberg et al. and Walt et al. The motivation to do so, allegedly provided by Nyren et al., would have been that using protected nucleotides allowed chain extension to proceed one position at a time without complications caused by

sequences of identical bases. Office Action mailed 12/12/06: p.12; Office Action mailed 5/30/07: p.15.

In rejecting claims 18, 19, 28-30, 43 and 48 as being unpatentable over Rothberg et al., supra, Walt et al., supra, Nyren et al., supra, and Stratagene Catalog (1988, at page 39) ("Stratagene Catalogue"), the Examiner has made the following assertions on the record, inter alia, in Office Actions mailed 12/12/06 and 5/30/07, to meet the Examiner's burden of establishing a prima facie obviousness rejection.

(1) Regarding claims 18, 19, 28-30, 43 and 48, the Examiner has taken the position that Rothberg et al. teach each component of the claimed kits for nucleic acid sequencing *except* the kit format itself and the use of microspheres to attach sequencing reactants to the surface of the fiber optic bundle. Office Action mailed 12/12/06: pp.12-14; Office Action mailed 5/30/07: pp.15-17.

(2) The Examiner further asserts that the alleged deficiency of Rothberg et al. with regard to base claim 18 is cured by Walt et al., which is alleged to teach microsphere-based analytical chemistry system in which the microspheres are distributed on a fiber optic bundle that comprises discrete sites into which at least two subpopulations of micro spheres are distributed. According to the Examiner, each of the microspheres described by Walt et al. comprises a bioactive agent and an optical signature which allows identification of the bioactive agent. The microspheres are allegedly randomly distributed on the array and the bioactive agent that is allegedly attached to the microsphere is a nucleic acid probe. The array is allegedly used for sequencing. The Examiner further alleges that Walt et al. teaches elements of claims 22, 31, 32, 35 and 38 that relate to the microspheres and are not taught by Rothberg et al. Regarding claim 43, Walt et al. are alleged to teach enzymes immobilized on microspheres. Office Action mailed 12/12/06: p.14; Office Action mailed 5/30/07: p.17.

(3) The Examiner claims that it would have been prima facie obvious to one skilled in the art to use the microspheres of Walt et al. distributed over the surface of a fiber optic sensor in the

sequencing method of Rothberg et al. allegedly because Walt et al. provide a method of generating large fiber optic arrays and because microsphere-based chemistry systems allow synthesis of bioactive agents to be separated from their placement on an array. (e.g., see also, Office Action mailed. 11/28/05: pp.10-11, and Advisory Action mailed 5/1/06: p.2). The motivation to use microspheres immobilized on a surface of fiber optic bundle rather than nucleic acids or proteins immobilized directly on the surface of the fiber optic bundle allegedly comes from Walt et al. since it allows for fast generation of large fiber optic arrays. Office Action mailed 12/12/06: pp.15-16; Office Action mailed 5/30/07: pp.17-19.

(4) The Examiner admits that neither Rothberg et al. nor Walt et al. teach kits. Office Action mailed 12/12/06: p.16; Office Action mailed 5/30/07: p.19.

(5) The Examiner alleges that Nyren et al. teach a kit for sequencing of DNA by pyrophosphate release. According to the Examiner, the kit includes a sequencing primer, a polymerase, a detection enzyme, means for identifying pyrophosphate release, dNTPs or ddNTPs. Office Action mailed 12/12/06: p.16; Office Action mailed 5/30/07: p.19.

(6) According to the Examiner, the Stratagene Catalog provides a motivation to combine reagents of use in an assay into a kit. Office Action mailed 12/12/06: p.16; Office Action mailed 5/30/07: p.19.

In rejecting claims 20 and 21 as being unpatentable over Rothberg et al., supra, Walt et al., supra, Nyren et al., supra, and Stratagene Catalog, supra, and further in view of Ross et al., WO 91/06678, the Examiner has made the following assertions on the record, inter alia, in Office Actions mailed 12/12/06 and 5/30/07, to meet the Examiner's burden of establishing a prima facie obviousness rejection.

(1) The Examiner relies upon the teachings of Rothberg et al. , Walt et al. , Nyren et al. and Stratagene Catalog as described above, but admits that none of these references teaches

labeled nucleotides or different labels on nucleotides. Office Action mailed 12/12/06: pp.16-17; Office Action mailed 5/30/07: pp.19-20.

(2) Ross et al. allegedly teach sequencing of nucleic acids by sequential addition of 3'–blocked nucleotides to the template wherein the nucleotides carry different labels Office Action mailed 12/12/06: pp.16-17; Office Action mailed 5/30/07: pp.19-20.

(3) According to the Examiner, it would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used labeled nucleotides of Ross et al. in the sequencing kit of Rothberg et al., Walt et al., Nyren et al. and the Stratagene Catalog. The motivation to do so, allegedly derived from Ross et al., is that incorporation of nucleotides was monitored by detecting the label on the dNTP and using fluorescent labels increased detection sensitivity. Office Action mailed 12/12/06: pp.16-17; Office Action mailed 5/30/07: pp.19-20.

B. Appellants' Rebuttal of the Rejections Under 35 U.S.C. 103(a)

1. Obviousness

A claimed invention is unpatentable if the differences between it and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the pertinent art. 35 U.S.C. § 103(a) (2000); Graham v. John Deere Co., 383 U.S. 1, 13-14 (1966). The U.S. Court of Appeal for the Federal Circuit recently re-iterated the proper standards for making determinations under § 103. In re Kahn, 441 F.3d 977 (Fed. Cir. 2006). First, the scope and content of the prior art is determined, the differences between the prior art and the claims at issue are ascertained along with the level of ordinary skill in the pertinent art. Against this background, a determination is made whether the subject matter would have been obvious to a person of ordinary skill in the art at the time of the asserted invention. Id. at 985 (citing Dann v. Johnston, 425 U.S. 219, 226 (1976) and Graham v. John Deere Co., 383 U.S. 1, 13-14 (1966)).

> To reject claims in an application under section 103, an examiner must show an unrebutted prima facie case of obviousness . . . . On appeal to the Board, an Appellant can overcome a rejection by showing insufficient evidence of prima

facie obviousness or by rebutting the prima facie case with evidence of secondary indicia of nonobviousness.

Rouffett, 149 F.3d at 1355.

The Federal Circuit has further noted that "[m]ost inventions arise from a combination of old elements and each element may often be found in the prior art." Kahn at 986.

> However, **mere** identification **in the prior art of each element is insufficient to defeat the patentability of the combined subject matter as a whole**. Rather, a party alleging invalidity due to obviousness must articulate the reasons one of ordinary skill in the art would have been motivated to select the references and to combine them to render the claimed invention obvious.
>
> Id. at 986.

For the reasons set forth below, Appellants respectfully submit that the Examiner has presented insufficient evidence of prima facie obviousness. Furthermore, the Examiner has ignored secondary indicia of nonobviousness that successfully rebut any prima facie obviousness

"A reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant." In re Gurley, 27 F.3d 551, 553 (Fed. Cir. 1994); see KSR Int'l Co. v. Teleflex Inc., 127 S. Ct. 1727, 1739-40 (2007) (explaining that when the prior art teaches away from a combination, that combination is more likely to be nonobvious).

The U.S. Patent and Trademark Office is in accord with the case law and recently promulgated guidelines for Examiners in making obviousness determinations in view of the U.S. Supreme Court's decision in *KSR Int'l Co. v. Teleflex Inc.* Examination Guidelines for Determining Obviousness under 35 U.S.C. 103 in View of the Supreme Court Decision in KSR International Co. v. Teleflex Inc., 72 Fed. Reg. 57,526 (2007) ("Guidelines") One important feature of the Guidelines is an *explicit requirement* that an Examiner provide articulated reasons for the factual determinations underlying an asserted prima facie case of obviousness. This focus is consistent with the rule set down in the *KSR* decision that a factfinder must provide "reasons"

why an invention would have been obvious to one of ordinary skill in the art. ." *KSR* at 1741. In explicating this aspect of the Supreme Court's decision, the Guidelines set forth several different rationales that can be used to support an obvious rejection. The Guidelines further set forth explicit factual findings that an Examiner must articulate to support an obviousness rejection under each rationale. In the present case the Examiner has applied the "teaching, suggestion or motivation" test, identified in the guidelines as rationale (G). For an obviousness rejection based on this rationale for combining references, the Examiner *is required to articulate* the following: (1) a finding that there was some teaching, suggestion, or *motivation*, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to combine reference teachings; (2) a finding that there was reasonable expectation of success; and (3) whatever *additional findings based on the Graham factual inquiries* may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness. As described below, the Examiner has repeatedly failed to articulate a motivation to use microspheres in the pyrophosphate sequencing method of Rothberg et al. and has thus failed to establish a prima facie case.

2. APPELLANTS' REBUTTAL TO REJECTION OF CLAIMS 1-27, 31-38, 40-42, 44-47 AND 50 AS ALLEGEDLY UNPATENTABLE OVER ROTHBERG ET AL., SUPRA, AND WALT ET AL., SUPRA.

a. Alleged Motivation to Combine Omits Elements of Claimed Invention

Appellants respectfully point out that arguments have been presented on multiple occasions on the record supporting Appellants' contention that the Examiner has failed to articulate a prima facie case of obviousness beyond the mere reiteration of two paragraphs quoted from Walt et al., which fail to address Appellants' central argument. As previously articulated, the question regarding motivation necessarily must include the particular method of nucleic acid sequencing of Rothberg et al. (Appellants' Pre-Appeal Brief Request for Review, filed June 27, 2006). Absent inclusion of this element, the Examiner has failed to establish a prima facie case of obviousness because there is no showing of a motivation to combine the cited references to arrive at a method containing all claimed elements. In particular, Appellants stated:

> However, the question is not whether motivation exists to use beads on a fiber optic surface. Walt et al. describes such a use. Rather, the question is whether: [O]ne of ordinary skill in the art at the time of the invention [would have been motivated to use] the microspheres of Walt et al. distributed over the surface of the fiber optic sensor in the method of nucleic acid sequencing of Rothberg et al.

Appellants' Response filed Nov. 28, 2005, at p.10 (emphasis added).

> As the rejection stands, the Examiner has failed to articulate a prima facie case of obviousness because the motivation, teaching or suggestion has been completely omitted. The "mere identification in the prior art of each element is insufficient to defeat the patentability of the combined subject matter as a whole." *In re Kahn*, Case No. 04-1616, slip op. at 11 (Fed. Cir. March 22, 2006) (citing *In re Rouffet*, 149 F.3d 1350, 1355, 1357 (Fed. Cir. 1998)). As articulated, the mere mention of using beads on a fiber optic surface fails to provide the proper motivation to combine because it does nothing more than identify one element of the claim. (Advisory Action mailed May 1, 2006, at p.2).

Appellants' Response filed September 18, 2006, page 10, third paragraph through page 11, second paragraph (emphasis original).

Appellants maintain that a mere description of using beads in an array that omits any reason for using the beads in an array for the nucleic acid sequencing method described by Rothberg et al. fails to give rise to a proper motivation for using microspheres in the claimed invention Under a proper obviousness inquiry, the requisite motivation or other rationale must include the nucleic acid sequencing element as is claimed by the invention. Appellants maintain that the identification of using beads in an array in the cited references without articulating a motivation or any other rationale to use of microspheres in the pyrophosphate sequencing method of Rothberg et al. is insufficient to establish a prima facie case.

b. Rothberg et al. Teach Away from Appellants' Claimed Invention

Rothberg et al. explicitly point to reported problems associated with the use of pyrophosphate sequencing in combination with beads s (see, for example, Appellants' Response filed December 19, 2005, at page 11, para. 2; Pre-Appeal Brief Request for Review filed June 27, 2006, at page 2, para. 3 through page 3, para. 3, and discussion further below). The Examiner's contention that Walt et al. supply the element of microspheres for use in a pyrosequencing method does not overcome Rothberg et al.'s teaching away from bead usage in pyrosequencing methods. There is nothing in Walt et al., that addresses, much less contradicts, Rothber et al.'s

explicit teaching away. One skilled in the art would not have been motivated to combine microspheres with the Rothberg et al. method of pyrosequencing in view of the express teaching away from such a combination in Rothberg et al. due to the expressed problem of microsphere and sample loss limitations.

Rothberg et al. clearly communicate to the skilled person that combining pyrophosphate sequencing with microspheres is undesirable. Appellants' Response filed December 19, 2005. For example, Rothberg et al. explicitly point to reported problems associated with the use of pyrophosphate sequencing in combination with microspheres in their characterization of Ronaghi et al. as being undesirable:

> In these early studies, sequencing of a PCR product using streptavidin-coated magnetic beads as a solid support was presented. However, it was found that the loss of the beads during washing, which was performed between each nucleotide and enzyme addition, was the limiting factor to sequence longer stretches.

Column 21, lines 14-34.

In addition to the explicit statement cited above, other descriptions in Rothberg et al. clearly teach away from using beads in a microarray (*e.g.*, see Appellants' Responses dated December 19, 2005, at pp.11-12, and April 14, 2006, at pp.10-12). Rothberg et al. also discuss at length possible solutions to the problem of lateral diffusion of released pyrophosphate (PPi), which requires that anchor primers to be spaced no closer than 50 μm apart. (Rotheberg et al., Column 28:36-41). Rothberg propose five solutions that mitigate the signal diffusion so as to allow a higher density of anchor pads. (Rothberg et al., Columns 27:37-28:41). Rothberg et al. fail to mention the use of microspheres or even microspheres in wells as a possibility to reduce PPi diffusion (*e.g.*, see Rothberg et al., Columns 27:37-28:41, Appellants' Responses dated December 19, 2005, at pp.11-12, and April 14, 2006, at pp.12-14). Rather, Rothberg et al. conclude that the preferred solution to the diffusion problem was creation of a physical barrier to lateral diffusion by submerging the anchor primers into cavities "required" to be at least 50 μm in depth (*e.g.*, see Rothberg et al., Column 37:24-28; Appellants' Response dated April 14, 2006, at pp.12-13). Rothberg et al. do not describe cavities comprising beads (*e.g.,* see Appellants' Responses dated December 19, 2005, at pp.11-12, and April 14, 2006, at pp.12-14). Rothberg et al., despite being aware of Walt's optical sensor arrays as evidenced by their citation to Walt's own work, Michael et al., for describing a method of acid-etching cavities, clearly elected not to include placement of Michael et al.'s beads into their proposed cavities for use as a support (*e.g.*,

see Rothberg et al., Column 20:30-33, Appellants' Response dated April 14, 2006, at pp.12-13). Walt et al. does not address the problem of diminished detection and signal resolution for array surface formats due to PPi diffusion and certainly does not provide any solution to address Rothberg et al.'s discouragement of bead usage.

In summary, Rothberg et al. does not teach or suggest placing beads in wells. Rather, Rothberg et al. describes making deep cavities that physically prevent lateral diffusion of released pyrophosphate (PPi) from the sequencing reaction. Walt et al. can be viewed as nothing more than supplying an element because Walt et al. is directed to making arrays without concern for detecting diffusible substrates on such arrays and without providing the motivation that would overcome Rothberg et al.'s explicit discouragement of bead usage. Therefore, Rothberg et al. explicitly teaches away from using beads as an array support in pyrophosphate sequencing reactions. The mere identification of using beads by Walt et al. does not overcome this teaching away.

c. The Inventors' Admissions Confirm Appellants' Interpretation of Rothberg et al. as Teaching Away

In addition to the express instances of teaching away described above that Appellants have consistently pointed to on the record, Rothberg et al. explicitly state and advocate for the purposes of gaining an allowance in a continuation-in-part (CIP) application that bead loss was a significant problem in pyrophosphate sequencing methods. The Rothberg et al. CIP application, U.S. Serial No. 09/814,338, is attached as Exhibit A to Appellants Response filed March 8, 2007, and claims priority to the cited Rothberg et al. `320 patent.

The cited `320 Rothberg et al. patent was filed September 16, 1999. The only mention of beads employed in pyrosequencing methods is the language Appellants show teaches away from the claimed invention because it teaches that bead loss was a limitation to this method. This teaching away can be found in the `320 Rothberg et al. patent at column 21, lines 14-34 (see, for example, Appellants' Response filed December 19, 2005, at page 11, para. 2). No other mention of beads can be found elsewhere in the document. Therefore, at the time the subject invention was made, Rothberg et al. taught away from beads in pyrosequencing methods and Appellants explicitly claim such use.

The CIP of the '320 patent, filed by Rothberg et al. on March 21, 2001, more than one year after the subject application's actual filing date, adds description of the use of beads in pyrosequencing methods and relies heavily on this element as admittedly critical to the inventiveness of the CIP claims in order to achieve an allowance. The relevant assertions, expert declarations and Examiner's reasons for allowance are summarized below.

Rothberg et al. expressly recognizes bead loss to be a limitation in pyrosequencing methods in the CIP application. The recognized language corresponds to Appellants' cited teaching away in the `320 patent and states:

> However, it was found that the loss of the beads during washing, which was performed between each nucleotide and enzyme addition, limited the technique to short sequences.

Exhibit A to Appellants Response filed March 8, 2007, at page 39, lines 22-24 (emphasis added); *see also*, the `320 Rothberg et al. patent at column 21, lines 30-34 and Appelants' Response filed December 19, 2005, at page 11, para. 2. This teaching away is confirmed during prosecution of the CIP application when Rothberg's Response states:

> [T]he application expressly recognizes the problem of bead/sample loss during the sequencing reaction (see, *inter alia,* page 37, lines 6-9, page 39, lines 22-24; and Figure 4).

Response filed April 23, 2004, attached as Exhibit C to Appellants' Response filed March 8, 2007, at page 20, para. 2 (emphasis added).

Therefore, both the Rothberg `320 patent and the Rothberg et al. CIP application contain an express recognition that bead loss was understood to be a limiting factor in pyrosequencing methods prior to the subject application's actual filing date. Rothberg et al. further expressly confirmed this teaching away during prosecution of the CIP application.

During prosecution of the CIP application, statements made in a declaration filed in support of the Response arguments, attached as Exhibit D to Appellants' Response filed March 8, 2007, also confirm the above acknowledgements. In particular, Dr. Margulies attests:

> The application expressly recognizes the problem of bead/sample loss during the sequencing reaction. This teaching is found, *inter alia,* in the originally filed

> application on p. 37, l. 6-9, p. 39, l. 22-24; and Fig. 4). . . . Accordingly, the claimed apparatus and substrate yield significantly improved results, which are not obtained with other sequencing systems . . .

Exhibit D to Appellants' Response filed March 8, 2007, Margulies Declaration executed April 20, 2004, at para. 17-18 (emphasis added). In a second declaration, Dr. Margulies also attests:

> [T]he claimed parameters for well diameter . . . and well depth . . . are not arbitrarily chosen parameters. Well depth is selected on the basis of a number of competing requirements in a nucleic acid sequencing application: (1) wells need to be deep enough for DNA-carrying beads to remain in the wells . . .

Exhibit E to Appellants' Response filed March 8, 2007, Margulies Declaration executed December 22, 2006, at para. 8.

Therefore, on two different occasions during prosecution of the CIP application Dr. Margulies, who is skilled in the art, attests that the teachings from the cited '320 patent expressly recognizes bead loss to be a problem in pyrosequencing methods. Dr. Margulies declarations further point to changes made in parameters of their substrate, such as well depth, as being important for allowing the use of beads in their pyrosequencing methods.

The Examiner clearly relied upon the assertions made by Rothberg et al's representatives and by Dr. Margulies in allowing the claims of the CIP. In the Examiner Reasons for Allowance, the Examiner stated:

> The diameter range, the depth, and well-depth of the claimed caviated fiber optic wafer . . . are deemed non-obvious over the general teaching provided for by Chee et al. (of record), based on Margulies Declaration, as each of the above-mentioned parameters are critical to the laminar flow of the reaction reagents . . . (see page 3 bottom paragraph to page 4, top paragraph; Margulies Declaration).

Notice of Allowability mailed February 7, 2007, at page 2 (emphasis added). Attached as Exhibit B to Appellants' Response filed March 8, 2007. Thus, a stated consideration by the Examiner in issuing the Notice of Allowance for the CIP application was the continued assertions by Rothberg et al. and supporting expert declarations that bead loss limited the previous sequencing methods in accordance with the teachings of the parent application which eventually issued as the cited '320 patent.

The newly included express language to the use of beads in pyrosequencing methods in the Rothberg CIP application and advocation that this discovery overcomes the problems associated with such previous methods shows that Rothberg et al. initially and in the disclosure cited against Appellants claims taught away from the use of beads. In particular, the new matter added to the Rothberg et al. CIP can be found at, for example, paragraphs [0120] and [0127]-[0137] and exemplary state:

> In various embodiments, some components of the reaction are immobilized, while other components are provided in solution. For example, in some embodiments, the enzymes utilized in the pyrophosphate sequencing reaction (e.g., sulfurylase, luciferase) may be immobilized if desired onto the solid support. Similarly, one or more or of the enzymes utilized in the pyrophosphate sequencing reaction, e.g., sulfurylase, luciferase may be immobilized at the termini of a fiber optic reactor array. Other components of the reaction, e.g., a polymerase (such as Klenow fragment), nucleic acid template, and nucleotides can be added by flowing, spraying, or rolling. In still further embodiments one more of the reagents used in the sequencing reactions is delivered on beads.

Exhibit A to Appellants' Response filed March 8, 2007, at page 31, lines 21-29 (emphasis added).Rothberg et al's inclusion of the above express teachings confirm they came into possession of the use of beads only after the filing date of the `320 Rothberg et al. patent cited against Appellants claims.

The above-described evidence strongly corroborates Appellants' showing that the Rothberg et al. `320 patent teaches away from the use of beads in a pyrosequencing method because the `320 patent contains only negative teachings whereas the CIP application was modified to add express language for the use of beads only after the filing of the earlier `320 patent, and more significantly only after filing of the instant application. Hence, Rothberg's change of direction and express admission that previous use of beads was problematic in order to achieve allowance shows that Rothberg only later discovered what Applicant of the instant application has maintained throughout the record. Namely, that the use of beads in pyrosequencing methods was unobvious over the art at least because the art taught away from this embodiment.

In light of the above remarks, Appellants maintain that the claims are unobvious over the cited combination of Rothberg et al. and Walt et al. Therefore, Appellants respectfully solicit reversal of this rejection.

3. APPELLANTS' REBUTTAL TO REJECTION OF CLAIM 17 AS ALLEGEDLY UNPATENTABLE OVER ROTHBERG ET AL., SUPRA, AND WALT ET AL., SUPRA, AS APPLIED TO CLAIM 10, AND FURTHER IN VIEW OF NYREN ET AL., WO 98/13523

Claim 17 is a dependent claim that incorporates all the elements of base claim 10. As indicated by the Examiner, except for the element of a protected nucleotide, the rejection is the same as that of claim 10, which is addressed above. Nyren et al. is merely cited for allegedly describing pyrophosphate sequencing kits. As such, Nyren et al. does not address any of the deficiencies of the primary references by Rothberg et al. and Walt et al. as addressed above. Specifically, Nyren et al. does not provide the missing reason to combine *pyrophosphate sequencing* with a solid support such as a microsphere nor does Nyren et al. overcome Rothberg et al's. teaching away from such a combination(see, for example, Response filed April 14, 2006, page 15, second paragraph through page 16, first paragraph). Absent a prima facie showing of motivation to combine the cited references to arrive at all claimed elements of the base claims, the further combination of references to additional elements fails to cure the deficiencies for a prima facie showing. Accordingly, for the reasons articulated in the above remarks with regard to the primary references, Appellants maintain that the claim 17 is unobvious over the cited combination of Rothberg et al., Walt et al. Therefore, Appellants respectfully solicit reversal of this rejection.

4. APPELLANTS' REBUTTAL TO REJECTION OF CLAIMS 18, 19, 28-30, 43 AND 48 AS ALLEGEDLY UNPATENTABLE OVER ROTHBERG ET AL., SUPRA, AND WALT ET AL., SUPRA, NYREN ET AL., SUPRA, AND STRATAGENE CATALOG (1988, AT PAGE 39)

With respect to the kit claims of claims 18, 19, 28-30 and 43, under 35 U.S.C. § 103(a) as allegedly obvious over Rothberg et al., Walt et al., Nyren et al. and the Stratagene Catalog, The Stratagene catalog does not address any of the deficiencies of the combination of references by Rothberg et al., Walt et al. and Nyren et al. and is merely cited for allegedly providing motivation to combine reagents into an assay into a kit. As such, the Stratagene catalog does not address any

of the deficiencies of the primary references by Rothberg et al., Walt et al. and Nyren eta al. as addressed above. Specifically, the Stratagene catalog does not provide the missing reason to combine *pyrophosphate sequencing* with a solid support such as a microsphere nor does the Stratagene catalog overcome Rothberg et al's. teaching away from such a combination (see, for example, Response filed April 14, 2006, page 15, second paragraph through page 16, first paragraph). Absent a prima facie showing of motivation to combine the cited references to arrive at all claimed elements of the base claims, the further combination of references to additional elements fails to cure the deficiencies for a prima facie showing. Accordingly, Appellants respectfully solicit reversal of this rejection.

5. APPELLANTS' REBUTTAL TO REJECTION OF CLAIMS 20 AND 21 AS ALLEGEDLY UNPATENTABLE OVER ROTHBERG ET AL., SUPRA, AND WALT ET AL., SUPRA, NYREN ET AL., SUPRA, STRATAGENE CATALOG, SUPRA, AND FURTHER IN VIEW OF ROSS ET AL., WO 91/06678.

Claims 20 and 21 are dependent claims that incorporate all the elements of base claim 18. As indicated by the Examiner, except for the element of a label, the rejection is the same as that of claim 18, which is addressed above. Ross et al. does not address any of the deficiencies of the combination of references by Rothberg et al., Walt et al., Nyren et al. and the Stratagene Catalog, and is merely cited for disclosing labeled nucleotides or different labels on nucleotides. As such, Ross et al. does not address any of the deficiencies of the primary references by Rothberg et al., Walt et al., Nyren et. al. and the Stratagene Catalog as addressed above. Specifically, Ross et al. does not provide the missing reason to combine *pyrophosphate sequencing* with a solid support such as a microsphere nor does the Stratagene catalog overcome Rothberg et al's. teaching away from such a combination (see, for example, Response filed April 14, 2006, page 15, second paragraph through page 16, first paragraph). Absent a prima facie showing of motivation to combine the cited references to arrive at all claimed elements of the base claims, the further combination of references to additional elements fails to cure the deficiencies for a prima facie showing. Accordingly, for the reasons articulated in the above remarks with regard to the primary references, Appellants maintain that the claims 20 and 21 are unobvious over the cited

combination of Rothberg et al. , Walt et al. , Nyren et al. and Stratagene Catalog, further in view of Ross et al.. For the foregoing reasons, Appellants respectfully solicit reversal of this rejection.

VII. CONCLUSION

The Examiner did not articulate a prima facie basis to deny patentability to the claimed invention under 35 USC §103(a) for lack of requisite realistic motivation. In addition, the Examiner has not given weight to the significant teaching away of Rothberg et al. as confirmed by Rothberg et al's own admissions during the prosecution of the cited patent's progeny applications. Appellants, therefore, submit that the Examiner's rejection of the appealed claims under 35 USC §103(a) is not procedurally or legally viable and, hence, solicit reversal thereof.

VIII. PRAYER FOR RELIEF

For the reasons discussed supra, Appellants submit that the Examiner's rejections under 35 USC §103(a) are factually and legally erroneous and, hence, solicit the Honorable Board to reverse the Examiner's rejections of the appealed claims.

To the extent necessary, a petition for an extension of time under 37 CFR. 1.136 is hereby made. Please charge any shortage in fees due under 37 CFR. 1.17 and 41.20, and in connection with the filing of this paper, including extension of time fees, to Deposit Account 502624 and please credit any excess fees to such deposit account.

Respectfully submitted,

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CLAIMS APPENDIX

1. A method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) providing an array comprising:

i) a substrate with a surface comprising discrete sites;

ii) a population of microspheres comprising at least first and second subpopulations, distributed at discrete sites on a surface of a substrate; and

iii) an enzyme attached at said discrete sites, wherein said enzyme is used to generate a signal from pyrophosphate;

b) providing a first hybridization complex comprising said first domain of a first target sequence and a first sequence primer, wherein said first hybridization complex is attached to said first subpopulation;

c) providing second hybridization complex comprising said second domain of a second target sequence and a second sequence primer, wherein said second hybridization complex is attached to said second subpopulation;

d) simultaneously extending said first and second primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primers, respectively;

e) detecting the release of pyrophosphate (PPi) with said enzyme attached at said discrete site within a common reaction chamber of said simultaneous extensions to determine the type of said first nucleotide added onto said first and second primers, respectively; and

f) determining sequences for said plurality of target nucleic acids.

2. A method according to claim 1 wherein at least said first hybridization complex is covalently attached to said first microsphere.

3. A method according to claim 1 wherein at least said first sequence primer is attached to said first microsphere.

4. A method according to claim 1 wherein said first and second hybridization complexes comprise:

a) said first and second target sequences;

b) said first and second sequence primers;

c) first and second capture probes, wherein said capture probes are covalently attached to said first and second microspheres, respectively.

5. A method according to claim 1, wherein said first and second hybridization complexes comprise said first and second target sequences, respectively, said first and second sequence primers, a first and second adapter probe, respectively, and first and second capture probes, respectively, covalently attached to said first and second microspheres.

6. A method according to claim 1 further comprising:

d) extending said first and second extended primers by the addition of a second nucleotide to the second detection position using said first enzyme; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said second nucleotide added onto said first and second primers, respectively.

7. The method according to claim 1 wherein said PPi is detected by a method comprising:

a) contacting said PPi with a second enzyme that converts said PPi into ATP; and

b) detecting said ATP using a third enzyme, wherein said enzyme attached at said discrete sites comprises said second enzyme or said third enzyme.

8. A method according to claim 7 wherein said second enzyme is sulfurylase.

9. A method according to claim 7 wherein said third enzyme is luciferase.

10. A method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) providing first hybridization complex comprising a first target sequence and a first sequencing primer that will hybridize to the first domain of said first target sequence,

b) providing a second hybridization complex comprising a second target sequence and a second sequencing primer that will hybridize to the second domain of said second target sequence, wherein said first and second sequencing primers are covalently attached to microspheres distributed at discrete sites on a surface of a substrate, said discrete sites having an attached enzyme used to generate a signal from pyrophosphate;

c) determining the identity of a plurality of bases at said target positions, wherein said determining comprises simultaneously extending said first and second sequencing primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primers, respectively; and

d) detecting the release of pyrophosphate (PPi) with said enzyme attached at said discrete sites within a common reaction chamber of said simultaneous extensions to

determine the type of said first nucleotide added onto said first and second sequencing primers, respectively.

11. A method according to claim l0 wherein said first hybridization complex and said second hybridization complex each comprise a capture probe.

12. A method according to claim 10 wherein said capture probe is a sequencing primer.

13. A method according to claim 10 wherein said determining comprises:

a) providing a sequencing primer hybridized to said second domain;

b) extending said primer by the addition of a first nucleotide to a first detection position using a first enzyme to form an extended primer;

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer;

d) extending said primer by the addition of a second nucleotide to a second detection position using said enzyme; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer.

14. The method according to claim 13 wherein said PPi is detected by a method comprising:

a) contacting said PPi with a second enzyme that converts said PPi into ATP; and

b) detecting said ATP using a third enzyme, wherein said enzyme attached at said discrete sites comprises said second enzyme or said third enzyme.

15. A method according to claim 14 wherein said second enzyme is sulfurylase.

16. A method according to claim 14 wherein said third enzyme is luciferase.

17. A method according to claim 10 wherein said determining comprises:

a) providing a sequence primer hybridized to said second domain;

b) extending said primer by the addition of a first protected nucleotide using a first enzyme to form an extended primer;

c) determining the identification of said first protected nucleotide;

d) removing the protection group;

e) adding a second protected nucleotide using said first enzyme; and

f) determining the identification of said second protected nucleotide.

18. A kit for nucleic acid sequencing comprising:

a) a composition comprising:

i) a substrate with a surface comprising discrete sites;

ii) a population of microspheres distributed on said sites; wherein said microspheres comprise different capture probes, wherein said array is configured for simultaneous contact of said different capture probes with a common reaction chamber; and

iii) an enzyme attached at said discrete sites, wherein said enzyme is used to generate a signal from pyrophosphate;

b) a first extension enzyme; and

c) dNTPs.

19. A kit according to claim 18 further comprising:

d) a second enzyme for the conversion of pyrophosphate (PPi) to ATP; and

e) a third enzyme for the detection of ATP, wherein said enzyme attached at said discrete sites comprises said second enzyme or said third enzyme.

20. A kit according to claim 18 wherein said dNTPs are labeled.

21. A kit according to claim 20 wherein each dNTP comprises a different label.

22. The method according to claim 1, wherein said substrate comprises discrete sites and said first and second subpopulations of microspheres are randomly distributed on said sites.

23. The method according to claim 22, wherein said discrete sites are wells, and said first and second subpopulations of microspheres are randomly distributed in said wells.

24. The method according to claim 10, wherein said substrate comprises discrete sites and said microspheres are randomly distributed on said sites.

25. The method according to claim 10, wherein discrete sites are wells, and said microspheres are randomly distributed in said wells.

26. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate comprises a fiber optic bundle.

27. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is selected from the group consisting of glass and plastic.

28. The kit according to claim 18, wherein said discrete sites are wells.

29. The kit according to claim 18 or 28, wherein said substrate is a fiber optic bundle.

30. The kit according to claim 18 or 28, wherein said substrate is selected from the group consisting of glass and plastic.

31. The method according to claim 1, wherein said microsphere array is decoded prior to providing first and second hybridization complexes.

32. The method according to claim 31, wherein said microspheres further comprise an identifier binding ligand that will bind a decoder binding ligand such that the identity and location of each microsphere can be determined.

33. A method according to claim 11 wherein said first hybridization complex and said second hybridization complex further comprise an adapter probe.

34. A method of sequencing a genome comprising:

a) amplifying a genome, thereby obtaining a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions;

b) providing an array comprising:

i) a substrate with a surface comprising discrete sites;

ii) a population of microspheres comprising at least a first and second subpopulation, distributed at said discrete sites; and

iii) an enzyme attached at said discrete sites, wherein said enzyme is used to generate a signal from pyrophosphate;

c) hybridizing sequencing primers to said first domains of said target sequences, wherein said hybridization complexes are attached to said microspheres;

d) simultaneously extending said primers by the addition of a first nucleotide to a first detection position using a first enzyme to form an extended primer; and

e) detecting the release of pyrophosphate (PPi) with said enzyme attached at said discrete sites within a common reaction chamber of said simultaneous extensions to determine the type of said first nucleotide added onto said primers; and

f) determining sequences for said genome.

35. The method of claim 1, wherein said enzyme attached at said discrete sites is attached to a microsphere.

36. The method of claim 1, wherein said first and second target nucleic acids comprise PCR amplification products.

37. The method of claim 1, wherein said first and second target nucleic acids comprise genomic DNA.

38. The method of claim 10, wherein said enzyme attached at said discrete sites is attached to a microsphere.

39. cancelled.

40. The method of claim 10, wherein said target sequences are covalently attached to said microspheres.

41. The method of claim 10, wherein said first and second target nucleic acids comprise PCR amplification products.

42. The method of claim 10, wherein said first and second target nucleic acids comprise genomic DNA.

43. The kit of claim 18, wherein said enzyme attached at said discrete sites is attached to a microsphere.

44. The method of claim 1, wherein said common reaction chamber comprises a flow cell.

45. The method of claim 44, further comprising washing unreacted nucleotides from said flow cell following said simultaneously extending said first and second primers.

46. The method of claim 10, wherein said common reaction chamber comprises a flow cell.

47. The method of claim 46, further comprising washing unreacted nucleotides from said flow cell following said simultaneously extending said first and second sequencing primers.

48. The kit of claim 18, wherein said common reaction chamber comprises a flow cell.

49. The method of claim 34, wherein said common reaction chamber comprises a flow cell.

50. The method of claim 49, further comprising washing unreacted nucleotides from said flow cell following said simultaneously extending said primers.

EVIDENCE APPENDIX

None.

RELATED PROCEEDINGS APPENDIX

None.

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